

- of a biological pathway in a cell of said cell type at a plurality of levels of a perturbation to said biological pathway;
- (c) forming a model environmental response as a combination of said one or more biological pathway responses, wherein each of said one or more biological pathway responses in said combination is subject to an independent scaling transformation;
- (d) determining the value of an objective function of the difference between said environmental response and said model environmental response; and
- (e) minimizing said determined value of said objective function by varying the scaling transformation of said one or more biological pathway responses to obtain best scaling transformations that minimize said determined value of said objective function;

wherein said combination of said one or more biological pathway responses subject to said best scaling transformations represents the biological pathways involved in the action of said drug in said cell type.

#### REMARKS

The specification has been amended, pursuant to 37 C.F.R. § 1.78(a)(2), to contain a reference to the prior application to which the instant application claims priority. The specification has also been amended to correct inadvertent typographical errors discovered by Applicants during review of the application. Claims 1-47 were pending in the instant application as originally filed. Claims 1-40 and 46-47 have been canceled as drawn to non-elected subject matter, without prejudice to Applicants' right to pursue the subject matter of these canceled claims in related patent applications. New claims 48-87 have been added to distinctly claim particular embodiments of the elected invention. Thus, upon entry of the instant amendment claims 41-45 and 48-87 will be pending. A courtesy copy of the pending claims, as amended herein, is attached hereto as Exhibit A.

The amendments are fully supported in the instant application as originally filed. In particular, the new claims are drawn to computer systems that identify biological pathways according to the methods described in the instant specification as originally filed and recited in allowed claims 1-7, 9-40 and 46-51 of prior U.S. Patent Application Serial No. 09/074,983 to which the instant application claims priority. Such computer systems are described and

enabled, *e.g.*, in Section 5.3.3 (page 57, line 6 through page 59, line 15) of the specification as originally filed. The specific methods executed by the claimed computer systems are also supported in the specification as indicated, *inter alia*, by the following table:

<u>Claims</u>	<u>Support</u>
48-49	page 48, line 35 to page 51, line 33
50-51	page 51, line 35 to page 57, line 4
52-53	page 41, line 27 to page 44, line 22
54-56	page 39, line 19 to page 41, line 72
57-58	page 37, line 26 to page 38, line 9
59	page 60, lines 12-16
60-62	page 78, lines 10-11; page 78, lines 23-33; page 89, line 33 to page 90, line 9
63-65	page 93, line 15 to page 94, line 4
66	page 94, line 24 to page 95, line 4
67-79	Section 5.4 (page 59, line 18 to page 78, line 3)
80	page 95, line 29 to page 97, line 10
81-82	page 99, line 20 to page 100, line 17
83	page 26, lines 21-28; page 27, lines 26-30; page 28, lines 17-19
84-85	Section 5.3.2 (page 48, line 27 to page 57, line 4)
86-87	page 14, lines 29-32

For all of the above reasons, therefore, the amendments presented herein do not constitute new matter. Entry of the above-made amendments into the file history of the above-captioned application is respectfully requested.

Respectfully submitted,

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Enclosure

**EXHIBIT A: PENDING CLAIMS**  
**(AS AMENDED BY PRELIMINARY AMENDMENT)**  
**IN**  
**U.S. PATENT APPLICATION SERIAL NO.: *To Be Assigned***  
**(ATTORNEY DOCKET NO. 9301-058)**  
**ENTITLED: "METHODS FOR IDENTIFYING PATHWAYS OF DRUG ACTION"**  
**BY: ROLAND STOUGHTON AND STEPHEN H. FRIEND**

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41. (Amended) A computer system for identifying biological pathways involved in the action of a drug in a cell type comprising a processor and a memory coupled to said processor, said memory encoding one or more programs, said one or more programs causing said processor to perform a method comprising the steps of:

- (a) receiving a drug response of said drug in said cell type, said drug response comprising measurements of a plurality of cellular constituents in a cell of said cell type at a plurality of levels of drug exposure;
- (b) receiving one or more biological pathway responses, each of said one or more biological pathway responses comprising measurements of cellular constituents of a biological pathway in a cell of said cell type at a plurality of levels of a perturbation to said biological pathway;
- (c) forming a model drug response as a combination of said one or more biological pathway responses, wherein each of said one or more biological pathway responses in said combination is subject to an independent scaling transformation;
- (d) determining the value of an objective function of the difference between said drug response and said model drug response; and
- (e) minimizing said determined value of said objective function by varying the scaling transformations of said one or more biological pathway responses to obtain best scaling transformations that minimize said determined value of said objective function;

wherein said combination of said one or more biological pathway responses subject to said best scaling transformations represents the biological pathways involved in the action of said drug in said cell type.

42. The computer system of claim 41 where said steps of receiving comprise making said drug response measurements and said biological pathway response measurements available in said memory.

43. The computer system of claim 41 wherein said forming a model drug response comprises adding said one or more biological pathway responses.

44. The computer system of claim 41 wherein said objective function comprises a sum of squares of the differences of said drug response and said model drug response at said levels of drug exposure, said model drug response being provided at said levels of drug exposure by transforming by said scaling transformations said levels of drug exposure to corresponding levels of perturbations to each of said biological pathways and by interpolating said biological pathway responses to said corresponding levels of perturbations.

45. The computer system of claim 41 wherein said minimizing comprises performing the Levenberg-Marquandt method.

48. (new) The computer system of claim 41 wherein the method performed by said processor further comprises the steps of:

- (f) determining an expected probability distribution of minimized determined values of said objective function, and
- (g) assessing the statistical significance of the minimized determined value of said objective function in view of the expected probability distribution of minimized determined values of said objective function.

49. (new) The computer system of claim 48 wherein the expected probability distribution of minimized determined values of said objective function is determined by:

- (i) randomizing the drug response with respect to the plurality of levels of drug exposure and randomizing the model drug response by randomizing the one or more biological pathway responses with respect to the plurality of levels of perturbation to the one or more biological pathways;
- (ii) determining a theoretical minimum values of the objective function of the difference between the randomized drug response and the randomized model drug response;
- (iii) minimizing said determined theoretical value of the objective function by varying the scaling transformations of the one or more randomized biological

- pathway responses to obtain scaling transformations that minimize said determined theoretical value of the objective function; and
- (iv) repeating steps (i) through (iii) to determine a plurality of theoretical minimum values,

wherein said plurality of minimum values forms said expected probability distribution of minimized values.

50. (new) The computer system of claim 41 wherein the method performed by the processor further comprises a step of verifying that said biological pathways are biological pathways involved in the action of said drug in said cell type by a method comprising selecting a model response that behaves most similarly to a combined drug-perturbation response, said combined drug perturbation response being provided by a method comprising measuring a plurality of cellular constituents in a cell of said cell type exposed simultaneously to one or more levels of said exposure to said drug and to one or more levels of perturbations in said one or more biological pathways,

wherein the model drug response is selected from the group consisting of:

- (i) a first model drug response comprising the combination of one or more biological pathway responses subject to the best scaling transformations evaluated at one or more first sums, each first sum being the sum of one of said one or more levels of drug exposure subject to said scaling transformations and one of said one or more levels of perturbations to said biological pathways.
- (ii) a second model drug response comprising one or more second sums, each second sum being the sum of said drug response evaluated at one of said one or more levels of drug exposure and said combination of said one or more biological pathway responses subject to the best scaling transformations evaluated at one of said one or more levels of perturbations to said biological pathways,

wherein said biological pathways are verified as biological pathways actually involved in the action of said drug in said cell type if the first model response is selected.

51. (new) The computer system of claim 41 wherein the method performed by the processor further comprises a step of assigning a cellular constituent present in said drug response to the one of said one or more biological pathways in which the biological pathway response of the cellular constituent subject to its best scaling transformation has the greatest correlation with the drug response of the cellular constituent.

52. (new) The computer system of claim 41 wherein said scaling transformations comprise transformations of said levels of drug exposure to corresponding levels of said perturbations to said biological pathways.

53. (new) The computer system of claim 52 wherein said transformations of said levels of drug exposure are by linear mapping.

54. (new) The computer system of claim 41 wherein said one or more programs further cause said processor to interpolate the measurements of cellular constituents of the biological pathway in said cell of said cell type at a plurality of levels of perturbation so that the one or more biological pathway responses are interpolated.

55. (new) The computer system of claim 54 wherein the interpolating comprises approximation by a sum of spline functions.

56. (new) The computer system of claim 54 wherein the interpolating comprises approximation by a Hill function.

57. (new) The computer system of claim 41 wherein the one or more biological pathways in the cell type are those biological pathways likely to be involved in the action of the drug in the cell type.

58. (new) The computer system of claim 41 wherein the one or more biological pathways are selected from a compendium of biological pathways present in the cell type.

59. (new) The computer system of claim 41 wherein the cell type is substantially isogeneic to *Saccharomyces cerevisiae*.

60. (new) The computer system of claim 41 wherein the cellular constituents comprise abundances of a plurality of RNA species present in the cell type.

61. (new) The computer system of claim 60 wherein the abundances of the plurality of RNA species are measured by a method comprising contacting a gene transcript array with RNA from a cell of the cell type, or with cDNA derived therefrom, wherein a gene transcript array comprises a surface with attached nucleic acids or nucleic acid mimics, said nucleic acids or nucleic acid mimics being capable of hybridizing with said plurality of RNA species or with cDNA species derived therefrom.

62. (new) The computer system of claim 61 wherein the measurements of cellular constituents in step (a) are provided by a method comprising contacting one or more gene transcript arrays (i) with RNA, or with cDNA derived therefrom, from a cell of said cell type that is exposed to said drug, and (ii) with RNA, or with cDNA derived therefrom, from a cell of said cell type that is not exposed to said drug, and

wherein said measurements of cellular constituents in step (b) are provided by a method comprising contacting one or more gene transcript arrays (i) with RNA, or with cDNA derived therefrom, from a cell of said cell type that is exposed to said perturbation to said biological pathway, and (ii) with RNA, or with cDNA derived therefrom, from a cell of said cell type that is not exposed to said perturbation to said biological pathway.

63. (new) The computer system of claim 41 wherein the cellular constituents comprise abundances of a plurality of protein species present in the cell type.

64. (new) The computer system of claim 63 wherein the abundances of the plurality of protein species are measured by a method comprising contacting an antibody array with proteins from a cell of the cell type,

wherein the antibody array comprises a surface with attached antibodies that are capable of binding with the plurality of protein species.



65. (new) The computer system of claim 63 wherein the abundances of the plurality of protein species are measured by a method comprising performing two-dimensional electrophoresis of proteins from a cell of the cell type.

66. (new) The computer system of claim 41 wherein the cellular constituent comprise activities of a plurality of protein species present in the cell type.

67. (new) The computer system of claim 41 wherein the one or more biological pathways in the cell type comprise biological pathways originating at one or more specific cellular constituents, and wherein the perturbations to the biological pathways are performed by a method comprising modifying the one or more specific cellular constituents.

68. (new) The computer system of claim 67 wherein the one or more specific cellular constituents are modified by a method comprising causing expression of the one or more specific cellular constituents under the control of a controllable expression system.

69. (new) The computer system of claim 67 wherein the one or more specific cellular constituents are modified by a method comprising controllable transfection of genes expression the one or more specific cellular constituents.

70. (new) The computer system of claim 67 wherein the one or more specific cellular constituents are modified by a method comprising controllably decreasing abundances of RNA species encoding the one or more specific cellular constituents in a cell of the cell type.

71. (new) The computer system of claim 70 wherein the method of controllable decreasing abundances of RNA species comprises exposing a cell of the cell type to ribozymes targeted to cleave the RNA species.

72. (new) The computer system of claim 67 wherein the one or more specific cellular constituents are modified by a method comprising controllably decreasing the rate of

translation of RNA species encoding the one or more specific cellular constituents in a cell of the cell type.

73. (new) The computer system of claim 72 wherein the method of controllably decreasing the rate of translation of RNA species comprises exposing a cell of the cell type to antisense nucleic acids or antisense nucleic acid mimics that hybridize to the RNA species or to DNA encoding the RNA species.

74. (new) The computer system of claim 67 wherein the one or more specific cellular constituents are abundances of protein species or activities or protein species, and wherein the one or more specific cellular constituents are modified by a method comprising controllably decreasing the abundances in a cell of the cell type.

75. (new) The computer system of claim 74 wherein the method of controllably decreasing the abundances comprises causing expression in a cell of the cell type of the one or more protein species as fusion proteins comprising the protein species and a degron, wherein the degron is controllable to increase the rate of degradation of the protein species.

76. (new) The method of claim 74 wherein the method of controllably decreasing the abundances comprises exposing a cell of the cell type to antibodies, wherein the antibodies bind to the protein species.

77. (new) The method of claim 67 wherein the one or more specific cellular constituents are activities of protein species, and wherein the one or more specific cellular constituents are modified by a method comprising controllably decreasing the activities in a cell of the cell type.

78. (new) The method of claim 77 wherein the method of controllably decreasing the activities comprises exposing a cell of the cell type to drugs which directly and specifically inhibit the activities of the protein species.

79. (new) The method of claim 77 wherein the method of controllably decreasing the activities comprises exposing a cell of the cell type to dominant negative mutant protein species, wherein the dominant negative mutant protein species are proteins inhibiting said activities.

80. (new) A computer system for identifying a more pathway specific drug candidate from an initial drug candidate comprising:

a processor, and

a memory coupled to said processor and encoding one or more programs

wherein said one or more programs cause said processor to perform a method comprising the steps of:

(a) identifying the biological pathways involved in the action of an initial drug candidate by a method comprising:

- (i) receiving an initial drug response of said initial drug candidate in a cell of a cell type, said initial drug response comprising measurements of a plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to the initial drug candidate,
- (ii) receiving one or more biological pathway responses, each of said one or more biological pathway responses comprising measurements of cellular constituents of a biological pathway in a cell of the cell type at a plurality of levels of perturbation to the biological pathway,
- (iii) forming a model initial drug response as a first combination of said one or more biological pathway responses, wherein each of said one or more biological pathway responses in said first combination is subject to an independent scaling transformation,
- (iv) determining the value of an objective function of the difference between said initial drug response and said model initial drug response, and
- (v) minimizing the determined value of the objective function by varying the scaling transformation of said one or more biological pathways in the first combination to obtain a first set of best scaling transformations that minimize the determined value of the objective function,

so that the combination of said one or more biological responses subject to the first set of best scaling transformation represents the biological response pathways involved in the action of the initial drug candidate;

- (b) identifying the biological pathways involved in the action of a modified drug candidate, said modified drug candidate having a modified structure of the initial drug candidate, by a method comprising:
  - (i) receiving a modified drug response of said modified drug candidate in a cell of the cell type, said modified drug response comprising measurements of a plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to the modified drug candidate,
  - (ii) forming a model modified drug response as a second combination of the one or more biological pathway responses, wherein each of said one or more biological pathway responses in said second combination is subject to an independent scaling transformation,
  - (iii) determining the value of an objective function of the difference between said modified drug response and said model modified drug response, and
  - (iv) minimizing the determined value of the objective function of the difference between said modified drug response and said model modified drug response by varying the scaling transformation of said one or more biological pathways in the second combination to obtain a second set of best scaling transformations that minimize the determined value of the objective function,

so that the combination of said one or more biological responses subject to the second set of best scaling transformation represents the biological response pathways involved in the action of the modified drug candidate, and

wherein said modified drug candidate is identified as a more pathway-specific drug candidate than said initial drug candidate if fewer biological pathways are identified in the action of said modified drug candidate than in the action of said initial drug candidate.

81. (new) A computer system for identifying one or more specific biological pathways that are involved in the action of a drug and that mediate side-effects of the drug, said computer system comprising:

a processor, and

a memory coupled to said processor and encoding one or more programs,

wherein said one or more programs cause said processor to perform a method comprising the steps of:

- (a) identifying the biological pathways involved in the action of a first drug by a method comprising:
  - (i) receiving a first drug response of said first drug in a cell of a cell type, said first drug response comprising measurements of a plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to the first drug,
  - (ii) receiving one or more biological pathway responses, each of said one or more biological pathway responses comprising measurements of cellular constituents of a biological pathway in a cell of the cell type at a plurality of levels of perturbation to the biological pathway,
  - (iii) forming a model first drug response as a first combination of said one or more biological pathway responses, wherein each of said one or more biological pathway responses in said first combination is subject to an independent scaling transformation,
  - (iv) determining the value of an objective function of the difference between said first drug response and said model first drug response, and
  - (v) minimizing the determined value of the objective function by varying the scaling transformation of said one or more biological pathways in the first combination to obtain a first set of best scaling transformations that minimize the determined value of the objective function,

so that the first combination of said one or more biological responses subject to the first set of best scaling transformation represents the biological response pathways involved in the action of the first drug;

(b) identifying the biological pathways involved in the action of a second drug by a method comprising:

- (i) receiving a second drug response of said second drug in a cell of the cell type, said second drug response comprising measurements of a plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to the second drug,
- (ii) forming a model second drug response as a second combination of the one or more biological pathway responses, wherein each of said one or more biological pathway responses in said second combination is subject to an independent scaling transformation,
- (iii) determining the value of an objective function of the difference between said second drug response and said model second drug response, and
- (iv) minimizing the determined value of the objective function of the difference between said second drug response and said model second drug response by varying the scaling transformation of said one or more biological pathways in the second combination to obtain a second set of best scaling transformations that minimize the determined value of the objective function,

so that the second combination of said one or more biological responses subject to the second set of best scaling transformation represents the biological response pathways involved in the action of the modified drug candidate; and

(c) identifying specific biological pathways involved in the action of the first drug that are different from those biological response pathways involved in the action of the second drug so that one or more specific biological pathways that are involved in the action of the first drug and that mediate side-effects of the first drug are identified.

82. (new) A computer system for identifying one or more specific biological pathways that are involved in mediating therapeutic efficacy for a disease or disorder, said computer system comprising:

a processor, and  
a memory coupled to said processor and encoding one or more programs,  
wherein said one or more programs cause said processor to perform a method comprising the steps of:

- (a) identifying the biological pathways involved in the action of a first drug by a method comprising:
  - (i) receiving a first drug response of said first drug in a cell of a cell type, said first drug response comprising measurements of a plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to the first drug,
  - (ii) receiving one or more biological pathway responses, each of said one or more biological pathway responses comprising measurements of cellular constituents of a biological pathway in a cell of the cell type at a plurality of levels of perturbation to the biological pathway,
  - (iii) forming a model first drug response as a first combination of said one or more biological pathway responses, wherein each of said one or more biological pathway responses in said first combination is subject to an independent scaling transformation,
  - (iv) determining the value of an objective function of the difference between said first drug response and said model first drug response, and
  - (v) minimizing the determined value of the objective function by varying the scaling transformation of said one or more biological pathways in the first combination to obtain a first set of best scaling transformations that minimize the determined value of the objective function,so that the first combination of said one or more biological responses subject to the first set of best scaling transformation represents the biological response pathways involved in the action of the first drug;
- (b) identifying the biological pathways involved in the action of a second drug by a method comprising:
  - (i) receiving a second drug response of said second drug in a cell of the cell type, said second drug response comprising measurements of a

plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to the second drug,

- (ii) forming a model second drug response as a second combination of the one or more biological pathway responses, wherein each of said one or more biological pathway responses in said second combination is subject to an independent scaling transformation,
- (iii) determining the value of an objective function of the difference between said second drug response and said model second drug response, and
- (iv) minimizing the determined value of the objective function of the difference between said second drug response and said model second drug response by varying the scaling transformation of said one or more biological pathways in the second combination to obtain a second set of best scaling transformations that minimize the determined value of the objective function,

so that the second combination of said one or more biological responses subject to the second set of best scaling transformation represents the biological response pathways involved in the action of the modified drug candidate; and

- (c) identifying specific biological pathways involved in the action of both the first and second drugs so that one or more specific biological pathways that are involved in the action of said first drug and mediate therapeutic efficacy for the disease or disorder are identified.

83. (new) A computer system for identifying biological pathways involved in the action of a drug in a cell type comprising

a process, and

a memory coupled to said processor and encoding one or more programs,

wherein said one or more programs cause said processor to perform a method that comprises determining the best scaling transformation of one or more biological pathway responses which minimize the value of an objective function of the difference between a provided drug response and a model drug response, wherein:



- (a) said one or more biological pathway responses are the product of a method comprising measuring cellular constituents of one or more biological pathways in a cell of said cell type at a plurality of levels of perturbation to said biological pathways;
- (b) said provided drug response is provided by a method comprising measuring a plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to said drug; and
- (c) said model drug response is represented as a combination of said one or more biological pathway responses, each of said one or more biological pathway responses in said combination being subject to an independent scaling transformation; and

wherein the combination of said one or more biological pathway responses subject to said best scaling transformations identifies the biological pathways involved in the action of said drug in said cell type.

84. (new) The computer system of claim 83 wherein said computer system assigns a statistical significance to the combination of said one or more biological pathway responses subject to said best scaling transformations, wherein the statistical significance is assigned by a method comprising:

- (a) obtaining an expected probability distribution of minimized values of the objective function; and
- (b) assessing statistical significance of an actual minimized value of the objective function in view of the expected probability distribution, wherein the actual minimized value of the objective function is determined from the provided drug response and the model drug response.

85. (new) The computer system of claim 84 wherein the expected probability distribution is obtained by a method comprising:

- (a) randomizing the drug response with respect to the plurality of levels of drug exposure;

- (b) randomizing the model drug response by a method comprising randomizing the one or more biological pathway responses with respect to the plurality of levels of perturbation to the one or more biological pathways;
- (c) determining a theoretical minimum value of the objective function by determining best scaling transformations of the one or more randomized biological pathway responses which minimize an objective function of the difference between the randomized drug response and the randomized model drug response; and
- (d) repeating steps (a) through (c), so that a plurality of theoretical minimum values is thereby determined,

wherein the plurality of theoretical minimum values forms the expected probability distribution.

86. (new) A computer system for identifying biological pathways involved in the effect of an environmental change upon a cell type, said computer system comprising:

- a processor, and
- a memory coupled to said processor and encoding one or more programs,
- a memory coupled to said processor and encoding one or more programs,

wherein said one or more programs cause said processor to perform a method that comprises determining the best scaling transformation of one or more biological pathway responses which minimize the value of an objective function of the difference between a received environmental response and a model environmental response, wherein:

- (a) said one or more biological pathway responses are the product of a method comprising measuring cellular constituents of one or more biological pathways in a cell of said cell type at a plurality of levels of perturbation to said biological pathways;
- (b) said received environmental response is provided by a method comprising measuring a plurality of cellular constituents in a cell of the cell type at a plurality of levels of exposure to said environmental change; and
- (c) said model environmental response is represented as a combination of said one or more biological pathway responses, each of said one or more biological

pathway responses in said combination being subject to an independent scaling transformation; and  
wherein the combination of said one or more biological pathway responses subject to said best scaling transformations identifies the biological pathways involved in the effect of said environmental change upon said cell type.

87. (new) A computer system for identifying biological pathways involved in the effect of an environmental change upon a cell type, said computer system comprising:

a processor, and

a memory coupled to said processor and encoding one or more programs,

wherein said one or more programs cause said processor to perform a method comprising the steps of:

- (a) receiving an environmental response to said environmental change upon said cell type, said environmental response comprising measurements of a plurality of cellular constituents in a cell of said cell type at a plurality of levels of exposure to said environmental change;
- (b) receiving one or more biological pathway responses, each of said one or more biological pathway responses comprising measurements of cellular constituents of a biological pathway in a cell of said cell type at a plurality of levels of a perturbation to said biological pathway;
- (c) forming a model environmental response as a combination of said one or more biological pathway responses, wherein each of said one or more biological pathway responses in said combination is subject to an independent scaling transformation;
- (d) determining the value of an objective function of the difference between said environmental response and said model environmental response; and
- (e) minimizing said determined value of said objective function by varying the scaling transformation of said one or more biological pathway responses to obtain best scaling transformations that minimize said determined value of said objective function;

wherein said combination of said one or more biological pathway responses subject to said best scaling transformations represents the biological pathways involved in the action of said drug in said cell type.